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B-cell activating factor (BAFF) plasma level at the time of chronic GvHD diagnosis is a potential predictor of non-relapse mortality

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Abstract

Biological markers for risk stratification of chronic GvHD (cGvHD) could improve the care of patients undergoing allogeneic hematopoietic stem cell transplantation. Increased plasma levels of B-cell activating factor (BAFF), chemokine (C-X-C motif) ligand 9 (CXCL9) and elafin have been associated with the diagnosis, but not with outcome in patients with cGvHD. We evaluated the association between levels of these soluble proteins, measured by ELISA at the time of cGvHD diagnosis and before the initiation of therapy, with non-relapse-mortality (NRM). Based on the log-transformed values, factor levels were divided into tertiles defined respectively as low, intermediate, and high levels. On univariable analysis, BAFF levels were significantly associated with NRM, whereas CXCL9 and elafin levels were not. Both low (≤2.3 ng/mL, hazard ratio (HR)= 5.8, P= 0.03) and high (>.7 ng/mL, HR=5.4, *P* =0.03) BAFF levels were associated with a significantly higher NRM compared with intermediate BAFF level. The significant effect of high or low BAFF levels persisted in multivariable analysis. A subset of cGvHD patients had

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persistently low BAFF levels. In conclusion, our data show that BAFF levels at the time of cGvHD diagnosis are associated with NRM, and also are potentially useful for risk stratification. These results warrant confirmation in larger studies.

Introduction

Chronic GvHD (cGvHD) remains the main long-term limitation to successful allogeneic hematopoietic stem cell transplantation (HSCT).[1–3] The signs and symptoms of cGvHD are often insidious. Thus, diagnosis and treatment typically occur after clinical manifestations are advanced. In addition, management of cGvHD may be suboptimal after discharge from the transplant center as patients are often followed by local oncologists unfamiliar with the clinical features of cGvHD.[4] Development of a much needed diagnostic and severity scoring system based on clinical manifestations of cGvHD is challenging because of the complex and pleomorphic nature of the disease.[5,6] In this context, validation of biological markers that would facilitate risk stratification and a more personalized approach to the management of cGvHD has the potential to improve outcomes.[1,6]

A number of potentially viable biomarkers in cGvHD have been identified through proteomics. Of these, soluble B-cell activating factor (BAFF), chemokine (C-X-C motif) ligand 9 (CXCL9) and elafin have been associated with the diagnosis of cGvHD[7–12] but their potential association with outcome remains unknown. In this study, we evaluate the association of these three soluble factors with non-relapse mortality (NRM) in a cohort of newly diagnosed cGvHD patients. We focused our assessment on the time of cGvHD diagnosis, before the initiation of systemic therapy for cGvHD, when clinical manifestations are potentially reversible and risk-adapted treatment is most likely to be successful.

Patients and Methods

Study population

From January 2007 through December 2010, 341 consecutive adult patients underwent their first allogeneic HSCT at the University of Michigan. Of these, a total of 287 survived beyond post-HSCT day (D100); 158 patients who developed cGvHD and had not been diagnosed with progression of their underlying malignancy before the onset of cGvHD were included in this analysis. All patients provided informed consent to participate in an institutional review board-approved, long-term follow-up study. Patient cGvHD data were prospectively collected into the University of Michigan Bone Marrow Transplant Program database.

Assessment of cGvHD and additional risk factors for NRM

Clinical cGvHD data were adjudicated by two clinicians with expertise in the field (CLK and DRC), and diagnosis and scoring were based on National Institutes of Health (NIH) Consensus Criteria.[5] Lung score calculation was based on results of pulmonary function tests performed within 4 weeks of the diagnosis of cGvHD per published recommendations.[13] The date of cGvHD diagnosis was defined as the time when NIH Consensus Criteria were first reached.

Previously reported risk factors for NRM after HSCT were examined.[14-18] Karnofsky Performance Status (KPS)[19] was assessed as >70 or ≤70%. Acute GvHD was scored by modified Glucksberg criteria.[20]

Measurement of potential protein biomarkers

ELISA was used to determine plasma concentrations of BAFF, CXCL9 and elafin as previously described.[9] Plasma samples were collected at the time of cGvHD diagnosis before the initiation of treatment for cGvHD and at 3 months interval thereafter. BAFF, CXCL9 and elafin levels were available in 112 (71%), 110 (70%) and 109 (69%) patients, respectively. All three protein levels were available for 105 of the 158 patients.

Statistical approach

NRM was defined as death because of any cause not related to progression or persistence of the underlying malignancy. NRM, overall survival (OS), progression-free survival (PFS) and progression of malignancy after cGvHD diagnosis were assessed by landmark analysis starting on the day of diagnosis of cGvHD. According to the inclusion criteria, none of the patients had experienced progression of the underlying malignancy before the onset of cGvHD. Therefore, in this study all reference to ‘progression of malignancy’ pertains to progression occurring after cGvHD diagnosis. Actuarial OS and PFS were estimated by the Kaplan-Meier method. The cumulative incidence method accounting for competing risks[21] was used to estimate the incidence of NRM (considering progression of the underlying malignancy or death with persistent malignancy as competing risks), and the incidence of progression of the underlying malignancy (considering NRM as a competing risk).

We evaluated the association between NRM and each of the plasma factors using Cox proportional hazards regression analysis on univariable and multivariable analysis. NRM-specific hazard ratios (HRs) were generated accounting for progression of the underlying malignancy or death with persistent malignancy as they occurred after cGvHD diagnosis.[22,23] Each plasma factor was initially evaluated in two separate Cox models in which the log-transformed values were divided into quartiles or into tertiles. The goodness-of-fit test (based on the Cox-Snell residuals) confirmed the superiority of the Cox model evaluating plasma values in tertiles. Therefore, all results pertaining to plasma factors were presented based on the tertile classification. The first, second and third tertiles were defined as low, intermediate and high levels, respectively.

To identify potential confounding factors in the association between plasma factors and NRM, we assessed patient sociodemographic, transplantation and GvHD-related factors as predictors of NRM. Patient, transplantation or cGvHD factors that were associated with NRM at the 0.003 (*P*-value adjusted based on Bonferroni correction to account for multiple comparisons) level on univariable analysis were considered in multivariable analysis. First-degree interaction effects were tested and were not found to be significant. Categorical variables were compared using the χ^2 and Fisher's exact tests and continuous variables were compared using the Wilcoxon rank-sum rest. Logistic regression analysis was used to assess the relationship between BAFF levels and patient sociodemographic, transplantation and cGvHD characteristics. The Wilcoxon matched-pairs signed rank test was used to compare

serial BAFF values. Unless otherwise noted, statistical significance was defined at the 0.05 level. Statistical analyses were performed primarily with the STATA software, version 11.0 (College Station, TX, USA).

Results

Chronic GvHD characteristics and potential causes of NRM

Chronic GvHD was diagnosed at a median of 189 days post HSCT (range 37-806, interquartiles 132-273). Twenty-one (13%) patients had early-onset cGvHD (before D100), including one case with biopsy-proven bronchiolitis obliterans syndrome. Organ and overall severity at the time of cGvHD onset are presented in Table 1. The baseline demographic, disease and transplant characteristics of the study cohort are described in Supplementary Table S1. No patient received antithymocyte globulin as part of the pre-HSCT regimen. As our cohort comprised patients identified at the time of first diagnosis of cGvHD, no treatments directed at cGvHD had been started yet. In this study, we did not capture therapies received before or after the day of blood draw when cGvHD was first diagnosed. We only captured the immunosuppressive therapy that patients were receiving on the day of blood draw when cGvHD was first diagnosed. Such therapy was related to GvHD prophylaxis or prior treatment of aGvHD. Immunosuppressive agents received on the day of diagnosis of cGvHD are summarized in Supplementary Table S1. Twenty-six patients (16%) were receiving a steroid, all but 2 (1%) at a dose of ≤ 0.5 mg/kg. Among the surviving patients, the median follow-up time from cGvHD diagnosis was 24 months (range 3–54). At the time of last follow-up, OS was 63% (95% confidence interval (CI) 51–73%), PFS 58% (95% CI 46–68%) and NRM 27% (95% CI 18–39). The causes of the 30 deaths in the cGvHD patient study cohort (N = 158) are listed in Supplementary Table S2.

Plasma factors as potential predictors of NRM

At the time of cGvHD diagnosis, median BAFF level was 3.6 ng/mL (range 0–19.4), median CXCL9 was 20 ng/mL (range 0–171) and median elafin was 8.8 ng/mL (range 1.5–36.5). The linear correlations among the three plasma factors are shown in Supplementary Table S3. Plasma BAFF levels were positively correlated with CXCL9 ($r = 0.42$, $P<0.01$), but not with elafin ($r = 0.18$, $P = 0.07$) levels.

On univariable analysis, BAFF levels at the time of cGvHD diagnosis were associated with NRM (Figure 1a), whereas CXCL9 (Figure 1b) and elafin (Figure 1c) levels were not (Table 2). NRM was significantly associated with either low or high BAFF level. Specifically, the 4-year cumulative incidence of NRM was 54% in the high BAFF level group and 30% in the low BAFF level group. In contrast, it was 11% in the intermediate BAFF level group (Figure 1a, $P = 0.02$). The NRM rates in the high and low BAFF level groups were comparable and not significantly different (HR = 1.0, $P = 0.9$).

The incidence of malignancy progression after cGvHD diagnosis was comparable across the three BAFF levels ($P = 0.7$) with a 4-year cumulative incidence of 22% (95% CI 10–46), 20% (95% CI 9–42) and 14% (95% CI 5–35) in the low, intermediate and high BAFF groups, respectively (Supplementary Figure S1a). Thus, the lower rate of NRM in the

intermediate BAFF group was not attributable to a higher rate of malignancy progression in this group of patients. The 4-year actuarial PFS in the low, intermediate and high BAFF groups was 25% (95% CI 2–61), 69% (95% CI 43–75) and 56% (35–72), respectively (Supplementary Figure S1b).

Patient, transplant and cGvHD-related characteristics did not differ between the subset of patients who were (n= 112) or were not (n =46) evaluable for BAFF assessment (data not shown) except for a higher prevalence (26% vs 11%, P=0.03) of progressive-onset cGvHD and a shorter time interval between transplantation and cGvHD diagnosis (median 167 vs 274 days, P<0.001) in the evaluable subset. These differences are unlikely to have biased our findings on the association between BAFF levels and NRM as neither one of these factors was associated with NRM (Supplementary Table S5) or with BAFF levels (Supplementary Table S6) in this patient population. Consistent with our data, a history of acute GvHD[10] and time to cGvHD onset[12] did not affect BAFF levels in independent patient cohorts. Importantly, NRM (HR = 0.96, P = 0.9), OS (HR = 1.2, P = 0.6) and PFS (HR = 1.3, P = 0.4) rates did not differ between patients who were or were not evaluable for BAFF assessment.

Impact of patient and transplantation characteristics on NRM and BAFF levels

In order to identify potential confounding factors in the association between BAFF levels and NRM, we evaluated patient, transplantation and GvHD-related characteristics in relation to NRM in the subset of patients (N=112) who were evaluable for BAFF assessment (Supplementary Tables S4 and S5). Our analysis revealed that KPS ≤ 70% (HR = 4.3, P = 0.001), and a history of severe (grades III–IV) aGvHD (HR = 5.5, P<0.001) were the only significant predictors of NRM. No other patient, transplant or GvHD characteristic examined was significantly associated with NRM. The results remained consistent when the univariate analysis was performed in the overall study cohort (N = 158). Multivariate analysis showed that low (HR = 8.5, P = 0.0009) and high (HR = 5.6, P = 0.03) BAFF levels remained significantly associated with NRM after adjustment for KPS ≤ 70% and a history of severe aGvHD (Table 3).

When we assessed the impact of patient and transplantation characteristics on BAFF levels, we found no significant association between low or high BAFF levels and patient age or gender, disease status at transplantation, intensity of conditioning regimen, donor type, GvHD prophylaxis regimen, a history of aGvHD, KPS, steroid therapy, platelet count and eosinophil count at the time of cGvHD diagnosis (Supplementary Table S6).These findings need to be interpreted with caution and validated in independent cohorts because our study was not powered for a systematic assessment of predictors of BAFF levels.

Influence of cGvHD characteristics on BAFF level

We considered whether cGvHD organ involvement, including skin, mouth, gut, liver, lung and eyes, was associated with BAFF levels. Of these, only skin involvement was significantly associated with BAFF levels (Figure 2). BAFF levels were significantly lower in patients with skin cGvHD than in patients without skin involvement (median 2.5 vs 5 ng/mL, P = 0.04), and patients with skin cGvHD were significantly more likely to have low

BAFF levels than patients without skin involvement (46% vs 20%, P=0.003). None of the other organs was individually associated with BAFF levels. Duration from transplantation to cGvHD diagnosis, cGvHD type and global NIH severity score at onset were not significantly associated with BAFF levels in this patient population.

Longitudinal assessment of BAFF before and after cGvHD onset

Finally, to determine whether high or low BAFF levels occur before cGvHD diagnosis, we examined BAFF levels before and serially after diagnosis of cGvHD. Among the 112 patients with samples available for BAFF measurement at the time of cGvHD diagnosis, baseline (measured at a median of 56 days (range 23–96 before the diagnosis of cGvHD, and at a median of 98 days post HSCT) BAFF level was obtainable for 39 (35%) patients. Baseline BAFF values were significantly lower (median 0.7 ng/mL, range 0.16–6, P=0.01) for patients who at the time of cGvHD diagnosis had a low BAFF level (n = 21) than for those who at the time of cGvHD diagnosis had an intermediate (n =8, median 1.7 ng/mL, range 0.35–16) or a high (n=10, median 1.98 ng/mL, range 0.9–5.8) BAFF level (Figure 3). BAFF levels seem to have spiked before cGvHD diagnosis for patients with intermediate or high BAFF levels at cGvHD onset. In contrast, for patients with a low BAFF level at cGvHD diagnosis, BAFF values remained at ~1 ng/mL before, at the time of and after cGvHD diagnosis. We were unable to determine whether persistently low levels of BAFF related to prior high-dose steroid administration because these data were not captured.

Discussion

The ability to predict NRM could guide therapeutic decisions in the management of cGvHD patients. The current study is the first one to demonstrate that soluble BAFF concentrations predict NRM in patients with cGvHD. A major strength of our study design is that BAFF concentrations were assessed at the time of diagnosis of cGvHD and before initiation of therapy. Validation of our findings in independent patient cohorts will be necessary before the risk stratification we are proposing based on BAFF concentrations could be used to guide therapy at the time of onset of cGvHD.

Our definition of BAFF levels was statistically derived; however, the ranges of BAFF concentrations in the low, intermediate and high BAFF groups respectively corresponded to BAFF concentrations reported in non-transplanted healthy individuals (median 1 ng/mL)[10,12,24–26], in patients with inactive cGvHD (median 5.7 ng/mL)[12] and in patients with active cGvHD (median 9.9 ng/mL)[12]. Of note, intermediate BAFF levels at the time of cGvHD diagnosis (median: 3.6 ng/mL, range 2.4–5.7 ng/mL) identified a group of patients at low risk for NRM. In contrast, BAFF concentrations on either side of the intermediate range were significantly associated with a high risk of NRM. We could not evaluate whether the association between BAFF levels and NRM is limited to patients with cGvHD because this study lacked a control group of transplant recipients who did not develop cGvHD. Hence, our findings apply to newly diagnosed cGvHD patients.

A significant association between elevated soluble BAFF levels and the diagnosis of cGvHD has consistently been shown in several patient cohorts,[9,10,12,27,28] and prior studies have shown an association between BAFF levels and cGvHD treatment response.[27,29] Consistent

with findings in prior studies,[10] BAFF levels in our current study were more likely to be elevated before the diagnosis of cGvHD in patients who presented with intermediate or high BAFF levels; and we have now linked persistently elevated BAFF levels to NRM in newly diagnosed cGvHD patients. Our finding that BAFF, but not two other cGvHD candidate biomarkers for cGvHD examined in this study (CXCL9 and elafin), was significantly associated with NRM further supports a role for BAFF in cGvHD pathobiology.[28,30,31] This finding is also consistent with work by others revealing that BAFF polymorphisms potentially associate with GvHD outcome after HSCT.[32] In contrast to patients who presented with intermediate or high BAFF levels, patients who presented with low BAFF levels at the onset of cGvHD tended to have persistently low levels before and after the diagnosis of cGvHD. This finding suggests that some patients with cGvHD have disease unaffected by BAFF.[33] Others have reported low BAFF levels in other inflammatory states such as inflammatory skin diseases including atopic dermatitis,[25,34] and recent data by Vital et al.[35] support a BAFF-independent inflammation pathway in the development of cutaneous systemic lupus erythematosus. In line with these data suggesting a BAFF-independent pathway in skin disorders, our data revealed that patients with low BAFF levels were significantly more likely to have skin cGvHD. The reported association between low BAFF and IgE mediated or related diseases[26] further support the need to further study the potential mechanism for development of organ-specific cGvHD manifestations in the context of low BAFF levels. Consistent with prior studies,[10] we did not detect significant associations between BAFF levels and other cGvHD, patient or transplantation characteristics.

The reason for the association between low BAFF levels and poor cGvHD outcome remains to be determined. Treatment with high-dose steroids was associated with lower BAFF levels in one study[10] but not in others.[3,8] In our evaluation, only a small fraction of the patient population was receiving steroid therapy at the time of cGvHD diagnosis, and we did not find an association of high-dose steroid use at the time of cGvHD diagnosis and BAFF level. This, however, does not preclude the possibility that patients with low BAFF levels had received prior steroid therapy for treatment of aGvHD. We were not able to verify this possibility in the current study because we did not capture the cumulative dose of steroids received before the diagnosis of cGvHD. High concentration of BAFF per B cell (BAFF/B-cell ratio) and promotion of activated B cells is found in patients with active cGvHD.[36,37] In contrast, supranormal B-cell numbers are associated with low BAFF levels. Herein, we were unable to directly assess whether low BAFF was related to high B-cell numbers because we did not evaluate B-cell reconstitution. Our data suggest attention is warranted in future studies to patients with low BAFF levels and cGvHD incidence and outcomes.

Because of its retrospective nature, this study had important limitations including the lack of data on the treatment of acute and chronic GvHD, and the lack of a control group of healthy controls and transplant recipients who did not develop cGvHD. However, in spite of these limitations and in light of prior data showing an association between BAFF levels and cGvHD treatment response,[27,29] our findings suggest that BAFF may aid in prognostication for cGvHD patients and merit validation in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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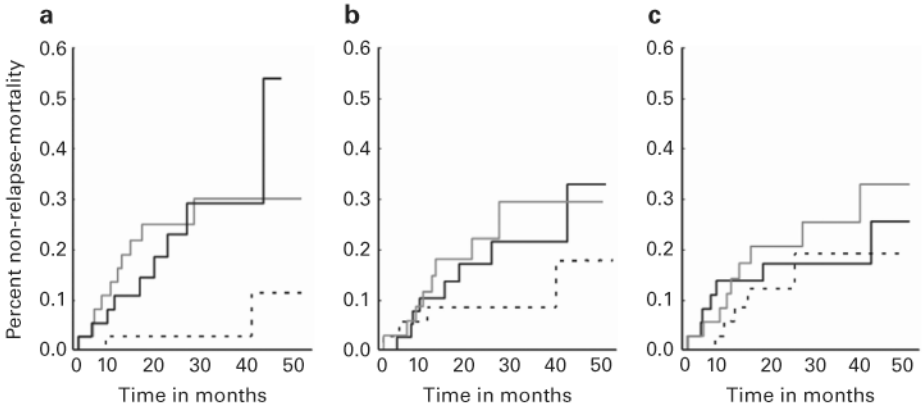

Figure 1.

Cumulative incidence curves of non-relapse mortality (NRM) after cGvHD diagnosis according to plasma concentrations at the time of cGvHD diagnosis of BAFF (**a**), CXCL9 (**b**) or elafin (**c**) in tertile groups. Concentration of each plasma factor measured by ELISA at the time of cGvHD diagnosis after allogeneic HSCT was divided into tertiles based on the log-transformed values. The first, second and third tertiles were defined as low, intermediate and high levels, respectively (as described in Statistical approach). The cumulative incidence of NRM curves are shown for cGvHD patients with high (gray line), intermediate (dotted black line) and low (bold black line) soluble factor levels as defined in Table 2.

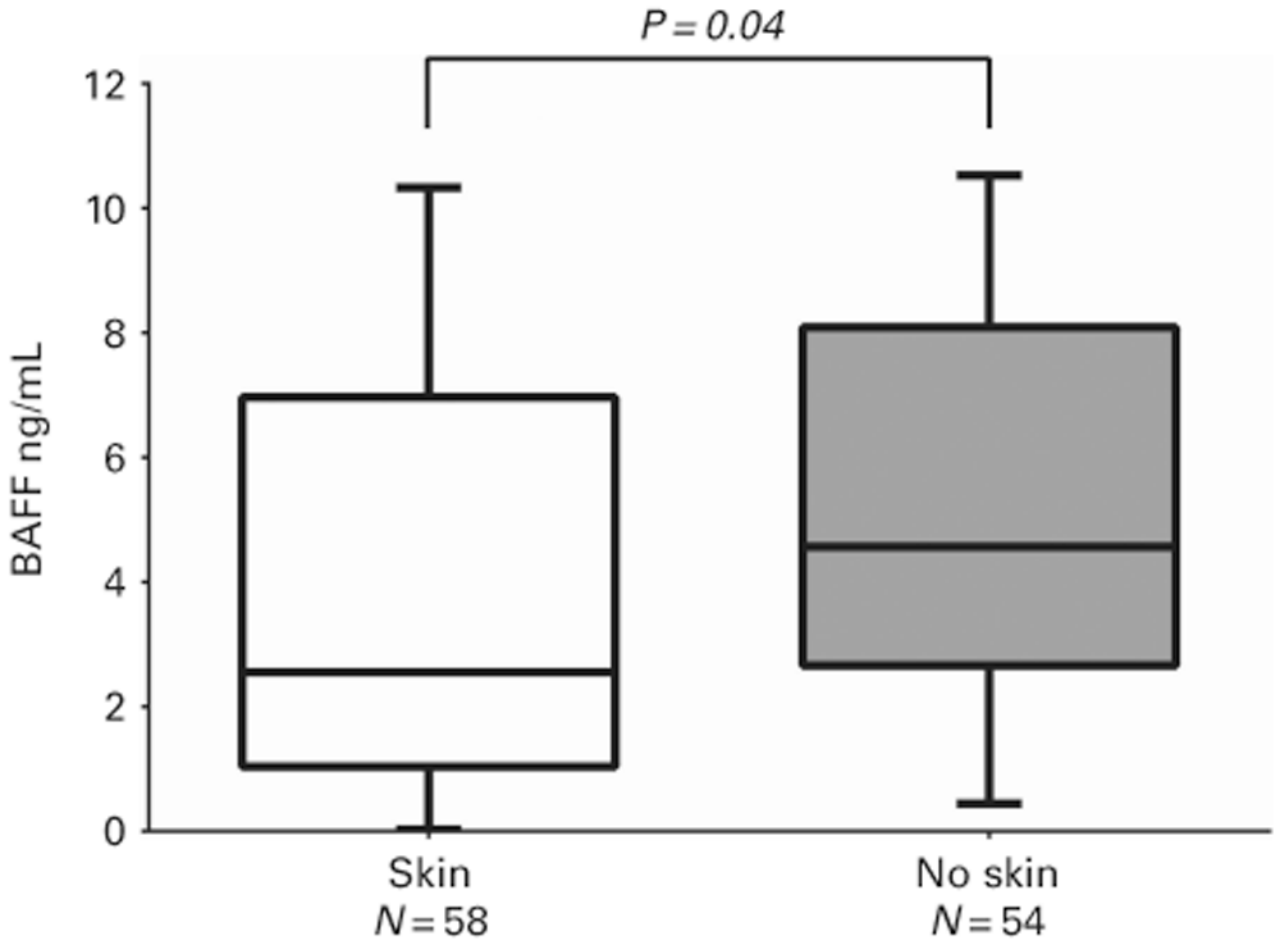

Figure 2.

Serum BAFF levels vary according to skin involvement in patients with cGvHD. Concentration of serum BAFF measured by ELISA at the time of cGvHD diagnosis after allogeneic HSCT in 58 patients with and 54 patients without skin involvement at the time of cGvHD diagnosis. Data are illustrated as box-and-whisker plots with the whiskers indicating the 90th and 10th percentiles. Lines within box plots indicate the median BAFF concentration for each group.

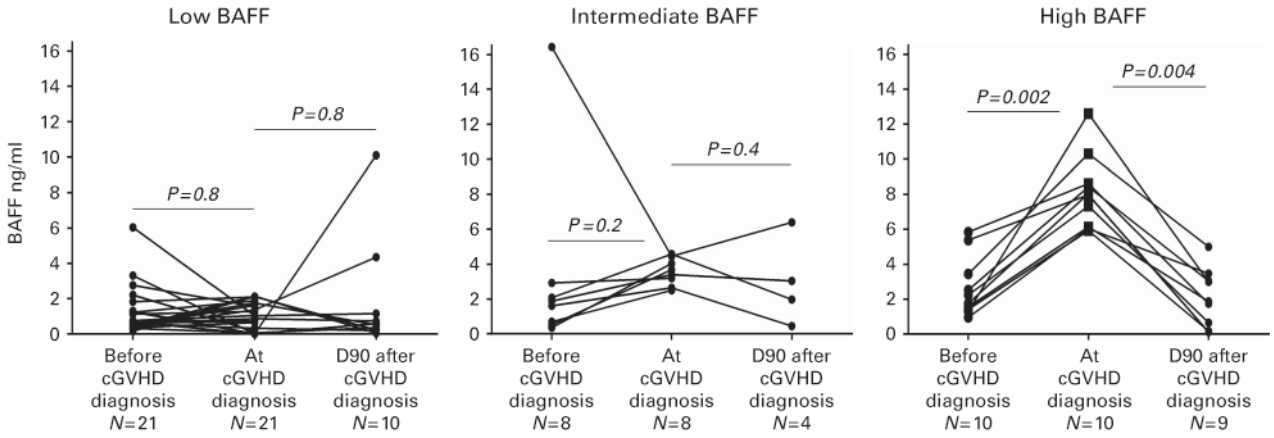

Figure 3.

Changes in plasma BAFF concentration over time in patients who had low, intermediate or high BAFF level at the time of cGvHD diagnosis. Concentrations of plasma BAFF measured by ELISA at the time of cGvHD diagnosis after allogeneic HSCT were divided into tertiles based on the distribution of the log-transformed values. The first, second and third tertiles were defined as low, intermediate and high levels, respectively (as described in Statistical approach). Serial ELISA results of plasma BAFF concentrations from cGvHD patients with high BAFF tertile (gray line), intermediate BAFF tertile (dotted black line) and low BAFF tertile (bold black line) are shown with the numbers (*n*) of samples available at each time point. *P*-values were derived based on the Wilcoxon matched-pairs signed rank test comparing BAFF values measured before, at the time of and after the diagnosis of cGvHD.

Table 1

GvHD characteristics at the time of cGvHD diagnosis of the study (*N*=158) cohort and of the subset of patients (*N*=112) evaluable for BAFF assessment

Characteristics	**N=*158***	***(%)***	**N = *112***	***(%)***
Days from transplant to cGvHD diagnosis, median (interquartiles)	189 (132,273)		167 (126,207)	
History of grade	34/40/20	21%/	29/24	26%/21%
I/II/III–IV aGvHD		25%/13%	/11	/10%
cGvHD onset presentation				
De novo	64	41%	48	43%
Quiescent	60	38%	35	31%
Progressive	34	21%	29	26%
Global NIH-CC severity				
Mild	23	15%	16	14%
Moderate	79	50%	54	48%
Severe	56	35%	42	37%
Organ involvement according to NIH-CC				
Skin				
None	78	49%	54	48%
Mild	32	20%	26	23%
Moderate	26	16%	16	14%
Severe	22	14%	16	14%
Mouth				
None	25	16%	12	11%
Mild	113	71%	85	76%
Moderate	18	11%	14	12%
Severe	2	1%	1	1%
Eye				
None	111	70%	80	71%
Mild	35	22%	25	22%
Moderate	11	7%	6	5%
Severe	1	1%	1	1%
Gastrointestinal				
None	128	81%	88	79%
Mild	25	16%	20	18%
Moderate	5	3%	4	4%
Liver				
None	68	43%	48	43%
Mild	40	25%	28	25%
Moderate	27	17%	16	14%
Severe	23	15%	20	18%
Lung (evaluable in 154/158 patients)				
None	63	41%	45	41%

Characteristics	**N=*158***	***(%)***	**N = *112***	***(%)***
Mild	74	48%	51	47%
Moderate	15	10%	11	10%
Severe	2	1%	2	2%
Joint				
None	148	94%	105	94%
Mild	9	6%	7	6%
Moderate	1	—	0	—

Abbreviations: aGvHD = acute GvHD; BAFF = B-cell activating factor; cGvHD = chronic GvHD;; NIH-CC = National Institutes of Health Consensus Criteria. The characteristics did not differ between the subset of patients who were (n = 112) or were not (n = 46) evaluable for BAFF assessment (data not shown)—except for a higher prevalence (26% vs 11%, P = 0.03) of progressive-onset cGvHD and a shorter time interval between transplantation and cGvHD diagnosis (median 167 vs 274 days, P< 0.001) in the evaluable subset.

Table 2

Univariable analysis of biologically relevant plasma soluble factors (*evaluated in tertiles*) and NRM

Soluble factor	N	*HR at 4 years*	*95% CI*	*P*-value
BAFF ng/mL				
0–2.3 (low)	38	5.8	1.2–27	0.03
>2.3–5.7 (intermediate)[a]	37	Reference		
>5.7–19.4 (high)	37	5.4	1.2–24	0.03
CXCL9 ng/mL				
0–10 (low)	39	2	0.7–6.7	0.25
>10–41 (intermediate)[b]	36	Reference		
>41–171 (high)	35	2.3	0.7–7.7	0.2
Elafin ng/mL				
1.5–6.3 (low)	37	1.3	0.4–4.05	0.7
>6.3–12.5 (intermediate)[c]	36	Reference		
>12.5–36.5 (high)	36	1.7	0.6–5.05	0.3

Abbreviations: BAFF = B-cell activating factor; CI = confidence interval; CXCL9 = chemokine C-X-C motif ligand 9; HR = hazard ratio; NRM = non-relapse mortality. Plasma concentration values of BAFF, CXCL9 and elafin were available in 112, 110 and 109 patients, respectively. Reference represents an hazard ratio (HR) of 1.0.

[a] Intermediate vs high or low BAFF (HR = 0.2, 95% CI 0.04-0.8, $P = 0.02$).

[b] Intermediate vs high or low CXCL9 (HR = 0.6, 95% CI 0.2-1.7, $P = 0.4$).

[c] Intermediate vs high or low elafin (HR = 0.7, (95% CI 0.2-1.9, $P = 0.4$).

Table 3
Multivariable analysis of predictors of NRM

Characteristic	*HR*	*95% CI*	P-*value*
Low BAFF levels	8.5	1.7–43	0.009
High BAFF levels	5.6	1.2–26	0.03
Maximum aGvHD severity 3 or 4	5.4	1.9–15	0.001
KPS ≤70%	2.6	1.02–6.5	0.045

Abbreviations: aGvHD =acute GvHD; BAFF = B-cell activating factor; CI = confidence interval; HR= hazard ratio; KPS=Karnofsky Performance Status; NRM = non-relapse mortality. Only patients (N=112) for whom plasma samples at the time of diagnosis of cGvHD were available were evaluable for multivariable analysis.